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09/593,914	06/14/2000	Jens J. Hyldig-Nielsen	BP9901US	8319

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EXAMINER

MYERS, CARLA J

ART UNIT PAPER NUMBER

1634

DATE MAILED: 07/17/2002

13

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

09/593914

Applicant(s)

Hyldig-Nielsen, J.

Examiner

Myers, C.

Group Art Unit

1634

--The MAILING DATE of this communication appears on the cover sheet beneath the correspondence address--

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, such period shall, by default, expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

- ☒ Responsive to communication(s) filed on 2/19/02
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

- ☒ Claim(s) 1-12, 16, 18-26, 29, 32, 33, 46-49, 60-62, 72, 80-87 is/are pending in the application.
- ☐ Of the above claim(s) _____ is/are withdrawn from consideration.
- ☐ Claim(s) _____ is/are allowed.
- ☒ Claim(s) 1-12, 16, 18-26, 29, 32-33, 46-49, 60-62, 72, 80-87 is/are rejected.
- ☐ Claim(s) _____ is/are objected to.
- ☐ Claim(s) _____ are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119 (a)-(d)

- ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been received.
- ☐ received in Application No. (Series Code/Serial Number) _____.
- ☐ received in this national stage application from the International Bureau (PCT Rule 1.7.2(a)).

*Certified copies not received: _____

Attachment(s)

- ☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____
- ☐ Notice of Reference(s) Cited, PTO-892
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Interview Summary, PTO-413
- ☐ Notice of Informal Patent Application, PTO-152
- ☒ Other Detailed Action

Office Action Summary

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1. This action is in response to Paper No. 12, filed January 17, 2002. Applicants arguments presented in the response of Paper No. 12 have been fully considered but are not persuasive to overcome all grounds of rejection. All rejections not reiterated herein are hereby withdrawn. This action is made non-final.

2. Applicant's election with traverse of Group I in Paper No. 12 is acknowledged. The traversal is on the ground(s) that undue burden would not be required to examine each of the inventions together because the inventions have the same classification and therefore the search for each invention is the same. This is not found persuasive because a search of invention I (SEQ ID NO: 1) would not lead one of skill in the art to references teaching inventions II-XI (SEQ ID NO: 2-11). A search of the distinct inventions would not be co-extensive as evidenced by the requirement for searching different keywords and nucleic acid sequences. Therefore, it is maintained that undue burden would be required to examine each of the claimed inventions.

Accordingly, the requirement is still deemed proper and is therefore made FINAL.

3. The response filed January 17, 2002 does not include a page "22" with amended claims 23, 24, and 33. While the "Marked-up" version of the amended claims has been used to examine the claims herein, in response to this Office action, Applicants are required to submit a complete "unmarked" version of the claims including amended claims 23, 24 and 33. Furthermore, claim 16 depends from canceled claim 15 and should be amended to depend from, for example, claim 9.

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4. Claims 46-49, 60-62 and 80-85 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A. Claims 46-49, 60-62 and 80-85 are indefinite over the recitation of "suitable *in-situ* hybridization conditions". While the specification discusses a number of different conditions which might constitute suitable conditions and discusses in general the concept of suitable *in situ* hybridization conditions, the specification does not provide a complete and fixed definition for this phrase. Furthermore, because the claim does not set forth the criteria for determining the suitability of the conditions and therefore it is unclear as to what would constitute suitable conditions. For example, it is unclear as to whether the conditions would allow for any *in situ* hybridization assay to be performed or if the conditions are specifically designed to allow for the *in situ* detection of yeast nucleic acids using an enzyme labeled probe.

Response to arguments:

In the response of Paper No. 12, Applicants traverse this rejection by stating that page 13 of the specification lists conditions which are suitable for achieving the desired degree of discrimination such that the assay generates an accurate and reproducible result. This argument is not convincing because the specification does not clearly define what is intended to be encompassed by the phrase "suitable *in-situ* hybridization conditions". While the specification states that "suitable *in-situ* hybridization conditions comprise conditions suitable for performing an *in-situ* hybridization process", the specification does not state that each recitation in the claims

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of "suitable in-situ hybridization conditions" corresponds to and is identical to a set of conditions set forth on page 13 of the specification which accomplish the result of achieving a specific degree of discrimination and of generating an accurate and reproducible result.

B. Claims 80-82 are indefinite because it is unclear as to whether the recited reagents of a)-h) are considered to be the "reagents or compositions necessary to perform the assay" or whether these reagents are present in addition to the "reagents or compositions necessary to perform the assay". It is also unclear as to whether the soybean labeled probe of e) is present in addition to the "one or more Dekkera/Brettanomyces specific probes" (as recited in a) of claim 80).

Response to arguments:

In the response of Paper No. 12, Applicants state that "the question need not be answered for the claim to be definite". This argument does not clearly address the rejection or clarify why applicants believe that the rejection is not proper. It is maintained that the claim is indefinite, vague and confusing because the claims are drawn to a kit comprising several sets of reagents and it is unclear as to whether the claims are intended to be limited to kits which contain each of these reagents or if the claims include only a portion of these reagents, with some of the reagents being further defined by the recitations in claims 80-82.

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person

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having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-9, 12, 20, 23, 24, 25, 26, 29, 32, 33, 46, 60, 72, 86, and 87 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosse in view of Stender (1998).

Kosse discloses dot blot and *in situ* hybridization methods for the detection and enumeration of *Dekkera bruxellensis*. Kosse teaches that the *in situ* hybridization method is performed using fluorescent labeled probes (e.g., TRITC or FLUOS) and that the dot blot hybridization is performed using digoxigenin labeled probes (page 469). The probes are complementary to sequences of the *Dekkera bruxellensis* 18S rRNA region (page 469; Table 2). The reference teaches that prior to *in situ* hybridization, yeast cell walls must be permeablized and that probes must be selected to yeast 18S rRNA regions which are fully accessible to probes (see page 478). Kosse teaches that *Dekkera bruxellensis* was successfully detected by *in situ* hybridization using 20% formaldehyde (see Table 2 and page 474). Table 2 lists additional 18S rRNA probes for the detection of other yeasts known to cause spoilage of dairy products (see page 468 and Table 2). Probes are also disclosed which are specific for all yeasts and for all eukaryotes (Table 2). Kosse further teaches that it is essential to provide accurate methods for

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detecting the presence of yeast in dairy products and other foods so as to ensure high quality and safe food products (see page 468).

Kosse teaches that the *in situ* hybridization method is performed using fluorescent-labeled probes and that dot blot hybridization is performed using digoxigenin labeled probes. Kosee does not specifically teach using enzyme-linked probes, or specifically soy bean peroxidase labeled probes.

However, Stender (1998) teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of Kosse so as to have specifically labeled the probes with soy bean peroxidase in order to have achieved the benefit of providing an effective means for labeling the probes, thereby facilitating the detection of *Dekkera bruxellensis*.

Secondly, Kosse does not teach immobilizing the probes on a solid support. However, Stender (1998) teaches that hybridization probes may be immobilized onto solid supports and particularly may be in the format of an array (page 31). It is stated that the use of an array provides the advantage of allowing for the simultaneous analysis using 100 or more different probes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have immobilized the probes of Kosse onto a solid support, as taught

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by Stender (1998), in order to have achieved the benefit of simultaneously assaying for the presence of distinct target sequences complementary to a multitude of different probes.

With respect to claim 25, Kosse does not teach adding blocking probes to the probe sets. However, Stender (1998; pages 25 and 28) teaches adding blocking probes (i.e., random non-selected probes) to hybridization reactions in order to reduce non-specific binding. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of Kosse so as to have included the "blocking probes" disclosed by Stender (1998) in order to have accomplished the objective of reducing non-specific binding of the yeast probes.

Additionally, Kosse does not teach packaging the probes into a kit. However, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the probes for detecting *Dekkera bruxellensis* and the reagents for detection assays in a kit for the expected benefits of convenience and cost-effectiveness for practitioners in the art wishing to detect *Dekkera bruxellensis*.

Response to arguments:

In the response of Paper No. 12, applicants traverse this rejection by stating that the cited prior art does not teach using enzyme labeled probes to detect yeast. Applicants point out that Stender teaches using enzyme-labeled probes to detect bacteria, but that there is no suggestion in the prior art to utilize the enzyme-labeled probes to detect yeast. Applicants further argue that Amann teaches away from the claimed invention because this reference teaches that because of

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the enzyme molecules size/weight, enzyme-labeled probes are not able to penetrate yeast cells and thus cannot be used to detect yeast by in-situ hybridization. Applicants arguments have been fully considered but are not persuasive to overcome the present grounds of rejection. Firstly, it is noted that the claims encompass probes that are "suitable for in-situ hybridization". Such probes would also be "suitable" for other types of hybridization assays, including dot-blot hybridization assays, as taught by Kosse. Accordingly, the teachings of Amann regarding methods of using enzyme-labeled probes for in-situ hybridization do not apply to probes that are to be used for other methods of hybridization. It is maintained that it would have been obvious to one of ordinary skill in the art at the time the invention was made to have labeled the probes of Kosse with enzymes, rather than fluorescent labels or with digoxigenin for the purposes of using the probes in methods such as dot blot hybridization because Stender teaches that enzyme labels may be used in place of fluorescent labels and provides an equally effective means for detecting hybridization of a probe to a target nucleic acids. Secondly, the claims drawn to methods do not require performing the steps of in-situ hybridization. Rather, the claims include methods of "hybridization" in general. The claims also include methods in which the hybridization conditions are generally stated to be "suitable for in-situ hybridization". The claims do not require any particular steps of in-situ hybridization and the conditions broadly recited in the claims include conditions for general methods of hybridization. Additionally, while Amann teaches that an enzyme-labeled probe was not useful for the detection of *Saccharomyces cerevisiae*, the reference teaches that modifying the conditions for permeabilization of cells

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allows one to use enzyme-labeled probes for some organisms. The specification provides no teachings as to critical steps that must be performed in order to allow for the detection of yeasts by in-situ hybridization using enzyme-labeled probes and the claims clearly do not recite any critical steps which distinguish the claims over prior art *in-situ* hybridization methods of detecting microorganisms using enzyme-labeled probes. Accordingly, given the teachings in the art of methods for detecting microorganisms using enzyme-labeled probes and the knowledge in the art of how to modify the conditions of permeabilization in order to allow for larger molecules to penetrate cells, the ordinary artisan would have had a reasonable expectation of success of applying the *in-situ* hybridization methods of Stender to the detection of yeast using enzyme labeled probes.

6. Claims 47-49 and 80-85 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosse in view of Stender (1998) and further in view of Parton (U.S. Patent No. 5,905,038).

The teachings of Kosse and Stender are presented above. The combined references do not teach filtering the microorganism and culturing the microorganism on a filter prior to performing in situ hybridization.

Parton (col. 1-2) teaches methods for isolating and analyzing samples for the presence of microorganisms wherein the methods comprise filtering a sample containing microorganisms through a membrane filter so as to trap the microorganisms on the filter and then culturing the microorganisms on the filter.

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In view of the teachings of Parton, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kosse in view of Stender so as to have isolated the microorganisms by filtration and then cultured the microorganisms on the filter prior to performing in situ hybridization in order to have provided an effective means for purifying and concentrating the microorganisms and for increasing the number of microorganisms to allow for the formation of colony forming units so as to increase the accuracy and sensitivity of the detection method.

Furthermore, Kosse and Stender do not teach kits comprising filters and culture media. However, the method of Kosse in view of Stender requires the use of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, and film. In view of the teachings of Parton, modification of the method of Kosse and Stender to include a filtration and culturing step would have resulted in a method which further required the use of a filter and culture media. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis*.

Response to arguments:

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In the response of Paper No. 12, Applicants traverse this rejection for the same reasons stated in paragraph 5 above. Accordingly, the response to those arguments applies equally to the present grounds of rejection.

7. Claims 1-12, 16, 18-26, 29, 32, 33, 46, 60-62, 72, 86 and 87 are rejected under 35 U.S.C. 103(a) as being unpatentable over De Wachter in view of Kosse and further in view of Stender et al (1998; reference BB).

De Wachter teaches an isolated nucleic acid consisting of the sequence of the 18S rRNA of *Dekkera/Brettanomyces bruxellensis*. The 18S rRNA of De Wachter comprises the sequence of SEQ ID NO: 1 (see nucleotides 1066-1052 of GenBank Accession No. X58052). The nucleic acid of De Wachter is considered to have the property of being suitable as a probe for the detection, identification or quantitation of *Dekkera/Brettanomyces bruxellensis*. De Wachter does not teach labeling the 18S rRNA with a detectable moiety, and particularly does not teach labeling the probe with an enzyme.

Kosse teaches hybridization methods, including dot blot hybridization and in situ hybridization, for the detection of *Dekkera bruxellensis*. Kosse teaches labeling probes with either chemiluminescent labels (e.g., digoxigenin) or fluorescent labels (e.g., TRITC or FLUOS) to facilitate the detection of yeasts and to particularly facilitate the detection of *Dekkera bruxellensis* (see page 469). The reference further exemplifies an 18S rRNA probe specific for *Dekkera bruxellensis* (see Table 2) and probes specific for other yoghurt spoiling yeasts. Kosse

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teaches that it is essential to provide accurate methods for detecting the presence of yeast in dairy products, such as yoghurt, so as to ensure high quality and safe food products (see page 468).

In view of the teachings of Kosse, it would have been further obvious to one of ordinary skill in the art at the time the invention was made to have used the labeled 18S rRNA of De Wachter as a probe under suitable hybridization conditions in order to have facilitated the detection of *Dekkera bruxellensis* in dairy samples. Additionally, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have generated probe sets comprising one or more probes for *Dekkera bruxellensis* or comprising probes for *Dekkera bruxellensis* and probes for other yeast and to have labeled each probe with a different detectable moiety in order to have allowed for the detection and differentiation of multiple types of yeast in dairy products, such as yoghurt.

The combined teachings of De Wachter and Kosse do not teach labeling the probes with soy bean peroxidase. However, Stender teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of De Wachter in view of Kosse by using soy bean peroxidase to label the probes in order to have provided an equally effective probe for the detection of *Dekkera bruxellensis*.

Secondly, De Wachter and Kosse do not teach immobilizing the *D. bruxellensis* and/or yeast probes onto a solid support. However, Stender teaches that hybridization probes may be

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immobilized onto solid supports and particularly may be in the format of an array (page 31). It is stated that the use of an array provides the advantage of allowing for the simultaneous analysis using 100 or more different probes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have immobilized the probes of De Wachter and Kosse onto a solid support, as taught by Stender, in order to have achieved the benefit of simultaneously assaying for the presence of target sequences complementary to a multitude of different probes.

Thirdly, the combined references do not teach PNA probes for the detection of *Dekkera bruxellensis*. However, Stender (see, for example, pages 3 and 10-11) teaches PNA probes complementary to rRNA sequences which are useful for the detection of microorganisms. Stender teaches that PNA probes hybridize to RNA or DNA with a higher affinity and specificity than their nucleic acid counterparts. PNA probes are also more stable due to their resistance to naturally occurring nucleases and proteases. Methods are disclosed for modifying nucleic acid probes so as to incorporate peptide nucleic acid moieties (see, for example, pages 13-14). Stender also teaches that PNA probes can be used in either *in situ* or *in vitro* hybridization methods (page 23). In view of the teachings of Stender, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of De Wachter and Kosse by including peptide nucleic acid moieties in the probes and thereby generating PNA probes, in order to have provided probes with increased affinity and specificity and increased resistance to nucleases and proteases.

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With respect to claim 25, De Wachter and Kosse do not teach adding blocking probes to the probe sets. Stender (page 25, 28) teaches adding blocking probes (i.e., random non-selected probes) to hybridization reactions in order to reduce non-specific binding. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of De Wachter and Kosse so as to have included the "blocking probes" disclosed by Stender in order to have accomplished the objective of reducing non-specific binding of the yeast probes.

With respect to claim 72, De Wachter does not teach packaging the 18S rRNA oligonucleotide in a kit. However, as discussed above, Kosse teaches using oligonucleotide probes for the detection of *Dekkera* and teaches the reagents required to perform hybridization assays. Furthermore, reagent kits for performing diagnostic methods were conventional in the field of molecular biology at the time the invention was made. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the *Dekkera bruxellensis* probes and reagents for performing hybridization in a kit for the expected benefits of convenience and cost-effectiveness for practitioners in the art wishing to detect *Dekkera bruxellensis*.

Response to arguments:

In the response of Paper No. 12, Applicants traverse this rejection for the same reasons stated in paragraph 5 above. Accordingly, the response to those arguments applies equally to the present grounds of rejection.

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8. Claims 47-49 and 80-85 are rejected under 35 U.S.C. 103(a) as being unpatentable over De Wachter in view of Kosse and Stender (1998) and further in view of Parton (U.S. Patent No. 5,905,038).

The teachings of De Wachter, Kosse and Stender are presented above. The combined references do not teach filtering the microorganism and culturing the microorganism on a filter prior to performing in situ hybridization.

Parton (col. 1-2) teaches methods for isolating and analyzing samples for the presence of microorganisms wherein the methods comprise filtering a sample containing microorganisms through a membrane filter so as to trap the microorganisms on the filter and then culturing the microorganisms on the filter.

In view of the teachings of Parton, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kosse in view of Stender so as to have isolated the microorganisms by filtration and then cultured the microorganisms on the filter prior to performing in situ hybridization in order to have provided an effective means for purifying and concentrating the microorganisms and for increasing the number of microorganisms to allow for the formation of colony forming units so as to increase the accuracy and sensitivity of the detection method.

Furthermore, the combined references do not teach kits comprising filters and culture media. However, the method of Kosse in view of Stender requires the use of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean

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peroxidase, and film. In view of the teachings of Parton, modification of the method of De Wachter, Kosse and Stender to include a filtration and culturing step would have resulted in a method which further required the use of a filter and culture media. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis*.

Response to arguments:

In the response of Paper No. 12, Applicants traverse this rejection for the same reasons stated in paragraph 5 above. Accordingly, the response to those arguments applies equally to the present grounds of rejection.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carla Myers whose telephone number is (703) 308-2199. The examiner can normally be reached on Monday-Thursday from 6:30 AM-5:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, W. Gary Jones, can be reached on (703)-308-1152. The fax number for the Technology Center is (703)-305-3014 or (703)-305-4242.

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Any inquiry of a general nature or relating to the status of this application should be directed to the receptionist whose telephone number is (703) 308-0196.

Carla Myers

July 15, 2002


CARLA J. MYERS
PRIMARY EXAMINER